Whole Exome Sequencing

Description:

The exome is comprised of all of the protein-encoding exons in the genome. Even though the exome accounts for only 1% of the entire genome, pathogenic variants in the exons account for many genetic disorders.

Exome sequencing (ES) examines the majority of exons and exon/intron boundaries of most of the genes at one time. This test is different from most genetic tests that only analyze one gene or a set of genes at a time. Approximately 25% of individuals who have exome sequencing receive a diagnosis or a suspected diagnosis from the test.

Indications:

- The patient's symptoms or family history suggest a genetic cause but do not align with a specific genetic disorder.
- The patient exhibits symptoms of a well-defined genetic disorder that has multiple genetic causes (genetic heterogeneity) for which a multi-gene panel is not clinically available.
- The patient likely has a genetic disorder, but previous clinical genetic testing failed to provide a diagnosis.
- The patient's clinical presentation is unclear or atypical, and there are multiple genetic conditions considered in the differential diagnosis.

What is Reported:

- Variants that are known to be pathogenic, or for which there is sufficient evidence suggesting pathogenicity, in a gene suspected to cause the patient's phenotype.
- Variants in a gene potentially related to the phenotype, but for which a specific clinical phenotype has not been previously well defined.
- Variants of uncertain clinical significance in genes related to the patient's phenotype.
- Variants that are pathogenic or for which the laboratory has sufficient evidence suggesting pathogenicity in certain medically significant genes unrelated to the patient's presenting symptoms unless the patient or parent/guardian declines this information.
- Only variants in the exons or exon/intron boundaries will be reported

What is Not Reported:

- Variants in genes not considered to be medically significant at the time of testing.
- Variants in genes that are unrelated to the proband's reported phenotype, with the exception of secondary findings (see below).
- Variants currently thought to be unrelated to any disease and that are common in healthy individuals.
- Variants predicting an increased risk of disease.
- Variants identified in research studies with an unclear relationship to disease.

Note: Family members submitting samples for comparative analysis will not receive a separate written report.

Secondary Findings:

For families who choose to receive secondary findings, a phenotype independent analysis of genes deemed medically actionable by the American College of Medical

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changing the outcome together

Genetics and Genomics Diagnostic Laboratory
CLIA#: 36D0656333
Phone: (513) 636-4474
Fax: (513) 636-4373
Email: LabGeneticCounselors@cchmc.org
www.cincinnatichildrens.org/genetics

Genetics and Genomics (Miller et al. 2023) will be performed for the proband only. In the instance of a positive finding, familial segregation information will be included in the patient's report, when known. Family members will not receive a separate report. Secondary findings will not be sought or reported if the patient or patient's representative chooses not to receive them.

Submission Requirements:

ES interpretation relies on the accuracy and completeness of the patient's clinical information including phenotype and family medical history. Simultaneous analysis of family members, such as a patient and parent trio, is likely to yield more informative results. The following items must be included to initiate the ES process:

- Proband's sample
- Maternal sample (for trio testing)
- Paternal sample (for trio testing)
- Additional family members' samples (following discussion with laboratory)
- Test requisition (all billing and clinical information must be completed)
- Signed informed consent form
- Family history and pedigree
- Detailed patient clinical history/clinical summary or medical notes
- Summary of previous genetic test results and reports, if available
- Letter of medical necessity describing how medical management will be impacted by the results of this test.

Methodology:

Procedure: Exome analysis is performed off a genome sequencing (GS) backbone. GS is performed on genomic DNA using a PCR-free library preparation and sequenced on an Illumina NovaSeq X Plus instrument to an average autosomal sequencing depth of at least 30x. Sequenced reads are aligned to human reference build (GRCh38) and variant calling is performed using the Illumina DragenGermline pipeline in AllCallers mode which produces simple variants (SNVs and small indels). Sample QC and identity are evaluated based on best practices for clinical whole-genome sequencing (Marshall et al. 2020). The sensitivity and specificity for SNVs and small insertions and deletions up to 50 base pairs is greater than 99%.

Data Analysis: Variant annotation and filtration are performed using a bioinformatics pipeline available in Fabric Enterprise. Variants are prioritized for review using GEM (Fabric Genomics) and classified for clinical significance based on the standards and guidelines for the interpretation of sequence variants, recommended by ACMG-AMP (Richards et al. 2015) with ClinGen rule specifications (https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation/). Reported variants are further limited to clinically relevant variants meeting our inclusion criteria. Carrier status for autosomal recessive conditions is not reported unless there is sufficient overlap with the reported clinical phenotype. Variants occurring in medically actionable genes may be reported for individuals who have elected to receive secondary findings. Reported variants are limited to pathogenic and likely pathogenic SNVs and small indels that meet reportable criteria in genes as defined by the ACMG Secondary Findings Maintenance Working Group (SFWG) (https://search.clinicalgenome.org/kb/genes/acmgsfpage=1&size=25&order=asc&sort=symbol&search=). Variant confirmation by an orthogonal technology is performed on reported variants unless otherwise indicated. Variants that have not been confirmed by an orthogonal methodology could represent technical artifacts.

Technical Limitations:

- ES attempts to examine approximately 20,000 genes in the MANE Select dataset. Each sample is required to achieve at least 20X coverage for 90% or more of the genomic regions occupied by these genes, based

on GS best practices (Marshall et al 2020). Regions with inadequate coverage will not be able to support accurate variant calls. Genes in the MANE Plus Clinical dataset that are not located on canonical chromosomes, unless manually curated (e.g., replacing the original transcript on a contig with a matched transcript on a canonical chromosome), are not supported by Fabric Enterprise software.

- Pathogenic variants may be present in a portion of the gene regions not covered by this test and therefore would not be identified. Thus, the absence of reportable findings for any gene does not mean there are no pathogenic variants in that gene.
- This analysis is limited to the detection of single nucleotide variants (SNVs), small deletions and insertions (indels). Examples of genomic abnormalities not detectable in the current version of this assay include cop number variations, mitochondrial genome variants, nucleotide repeat expansions (such as CGG repeats in FMR1, CAG repeats in HTT, etc.), balanced genomic rearrangements, absence of heterozygosity, and methylation differences. Variants at low level mosaicism or in challenging regions of the genome, (genes with pseudogenes, high GC content, repetitive regions, low sequencing coverage) may escape detection. Regions identified as systematically problematic due to high homology are listed in https://www.ncbi.nlm.nih.gov/books/NBK535152/.
- The clinical utility of ES depends on the accuracy of the clinical information provided by the referring physician. DNA sequencing from family members often improves the interpretation of test results.
- Due to the large number of variants called in each specimen, this assay utilizes the phenotype driven variant prioritization tool, GEM, by Fabric Enterprise V3 (De La Vega et al. 2021) as an automated first-tier variant filtration strategy. Disease associated loci may escape review prioritization for several reasons which include, but are not limited to, incomplete or inaccurate phenotype data, newly or yet to be established disease association, non-Mendelian etiology, poor variant quality metrics, or algorithmic bias. Additionally, GEM prioritization is limited to variants in genes with a MANE transcript ID (Matched Annotation from the NCBI and EMBL- EBI). Absence of identified variants does not rule out genetic causes.
- Our understanding of the human genome is incomplete at this time.
- Genetic changes identified may not predict severity or age of onset of a particular condition.

Post-test Counseling and Interpretation:

- It is highly recommended that patients have genetic counseling before the test is ordered, as they will have an important choice to make regarding which results they wish to know. Understanding the risks and benefits of this testing is important for the patient and his or her family. Genetic counseling after the test is likewise important to aid in the understanding of test results and their implications for the patient and his or her family members.
- It is the ordering physician's responsibility to interpret the results from this test within a clinical context.

Specimen:

At least 3 mLs whole blood in a lavender top (EDTA) tube. Label tube with patient's name, birth date, and date of collection. Alternately, 15 ug of DNA extracted from peripheral blood in a CLIA lab may be sent.

Turnaround Time:

- 84 days*

*Turn-around time for CCHMC patients will begin after prior authorization approval is received from patient insurance.

CPT Codes:

- Whole Exome Sequencing - Proband only: **81415**
- Whole Exome Sequencing - Trio: **81415, 81416x2**
- Whole Exome Sequencing - Extra Family Member: **81416**

For questions, please call 513-803-5390.

Shipping Requirements:

Please enclose test requisition and other required documents with sample. All information must be completed before sample can be processed.
Place samples in Styrofoam mailer and ship overnight at room temperature to arrive Monday through Saturday.*

Ship to:

Genetics and Genomics Diagnostic Laboratories
3333 Burnet Avenue TCHRF 1042
Cincinnati, OH 45229
513-636-4474

*For Saturday deliveries only: Please add "Dock 5" to the address and select the Saturday delivery check box on the shipping label if applicable.

Please call the lab at 513-636-4474 with shipment tracking information when available.

References:

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Clark, M. et al. (2018) Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. NPJ Genom Med. 2018 Jul 9;3:16.

De La Vega FM, et al. (2021) Artificial intelligence enables comprehensive genome interpretation and nomination of candidate diagnoses for rare genetic diseases. Genome Med. 13(1):153.

Kingsmore, S. et al. (2019) A Randomized, Controlled Trial of the Analytic and Diagnostic Performance of Singleton and Trio, Rapid Genome and Exome Sequencing in Ill Infants. Am J Hum Genet. 3;105:719-733.

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Miller, A. et al. (2023) ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 25(8):100866.

Richards S, et al. (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 17(5):405-424.